The epidermis: rising to the surface
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At the skin surface, the epidermis serves an important protective function which it manifests by building an extensive cytoskeletal architecture of keratin filaments, spanning from the nuclear envelope to hemidesmosomes and desmosomes. Recent studies on epidermal proteins and their interactions have provided insights into human skin diseases, including genetic disorders of keratins, laminins, and collagen. Explorations into the regulatory mechanisms underlying epidermal genes have underscored the importance of transcription factors AP-1 and AP-2, retinoic acid receptors, and POU proteins. Transgenic and gene ablation experiments on TGF-α and TGF-β genes have yielded clues as to how the epidermis maintains a balance of growing and differentiating cells.

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Introduction

The single-layered embryonic ectoderm receives mesenchymal cues which specify its programs of differentiation. Ectodermal proliferation begins laterally, with the mitotic plane perpendicular to the embryo surface. Shortly after stratification, condensates of dermal papilla (anlage) cells form in a dotted pattern beneath the embryonic basal layer. Where contact is made, basal cells begin to differentiate downward to craft what will ultimately be the hair follicles of the skin. In the absence of these mesenchymal–epithelial interactions, basal cells commit to an epidermal cell fate and stratify.

During these early stages as the skin surface undergoes rapid expansion, mitotic activity occurs in all layers, with the mitotic plane often parallel to the embryo surface. Later, as suprabasal cells begin to display morphological signs of differentiation, mitotic activity becomes restricted to a single layer of basal cells, with the mitotic plane reverting to a perpendicular orientation. This pattern persists in postnatal epidermis. In the adult, a basal cell responds to an as yet unidentified trigger of terminal differentiation. As it ceases to divide and begins its journey to the skin surface, it alters its adhesive properties, changes that are likely to be central to the control of the differentiative program (for a recent review, see [1*]). In transit, it undergoes a series of morphological and biochemical changes culminating in the production of dead flattened enucleated squames, which are sloughed from the surface and continually replaced by inner cells differentiating outward (Fig. 1). The trek takes two to four weeks time and continues throughout life. Here, we review recent advances in understanding the function and regulation of skin proteins.

Epidermal development and the program of terminal differentiation

Designing proteins that provide a protective barrier for the body: epidermal keratin filaments

The epidermis and its appendages devote the majority of their protein-synthesizing machinery to making keratins, which are α-helical proteins that assemble into 10 nm intermediate filaments. Two distinct sequence types of keratins first form coiled-coil heterodimers, which then pack laterally and end-to-end to form a single intermediate filament (for review, see [2*]). Type II keratins include K1–K8 (67–53 kDa) and the four Hb keratins of hair. Type I keratins include K9–K20 (63–40 kDa) and the Ha keratins of hair.

In skin, keratins are co-expressed as type I and type II pairs. Upon mesenchymal cues, ectodermal cells begin to express the basal epidermal keratins, K5 and K14, in a defined pattern over the body surface [3**]. As judged by expression of a K5 promoter driven β-galactosidase transgene, ectoderm overlying the somitic dermamyotome induces epidermal keratin transcripts as early as embryonic day E9.5 in mouse embryos, but it is not until E14.5 that the entire surface ectoderm expresses these genes (Fig. 2). In embryo and adult, K5 and K14 mRNAs seem to be restricted to cells that maintain their proliferative capacity ([3**] and references therein). As basal cells differentiate, they down-regulate expression of K5/K14 and induce new sets of differentiation-specific keratins (for review, see [2*]). Most body regions express K1 and K10 suprabasally (Fig. 1). K2e is also quite broadly expressed suprabasally, but its production is delayed relative to K1 and K10. K9 is confined to suprabasal palmar and plantar skin. K6 and K16 are

Abbreviations

Dsc—desmocollin; Dsg—desmoglein; E—embryonic day; EBS—epidermolysis bullosa simplex; EGF—epidermal growth factor; EH—epidermolytic hyperkeratosis; RAR—retinoic acid receptor; RXR—retinoid X receptor; TGF—transforming growth factor.

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unusual in that they are induced suprabasally during wound healing. These differences in keratin expression are regulated largely at the transcriptional level [4]. The functional significance of the multiplicity of keratins has not yet been resolved; however, the assembly properties of keratins differ as do their differential interactions with intermediate filament associated proteins.

**Blistering skin disorders of keratin: differentiation gone awry**

It has long been speculated that mutations in epidermal keratin genes cause skin diseases. A direct test of this hypothesis emerged when transgenic mice were engineered to express a truncated K14 gene in skin [5]. These mice displayed pathological features of a blistering human skin disease known as epidermolysis bullosa simplex (EBS), which affects \( \sim 1/50000 \) in the population and is transmitted in an autosomal dominant fashion. Basal EBS cells lyse in response to mild mechanical stress, thereby separating the epidermis from the underlying tissue (Fig. 1; Table 1). In the severest subtype, Dowling–Meara EBS, basal keratin exists as cytoplasmic clumps. Similar transgenic studies revealed that mice expressing a truncated K10 gene display features of epidermolytic hyperkeratosis (EH), a disease that resembles EBS, but which shows cytolysis and keratin clumps in suprabasal rather than basal layers (Table 1; for review, see [2*]). The degree of blistering correlates with the severity with which these mutants perturb intermediate filament assembly in vitro [6,7].

We now know that humans with EBS and EH have point mutations in either their K14 and K5 genes, for EBS [6,8,9], or their K1 and K10 genes, for EH [10–12]. K2e mutations are also predicted to cause EH cases with superficial blistering, and several groups are currently exploring this possibility. Patients with severe blistering frequently have mutations near the ends of the \( \alpha \)-helical 'rod' of the keratin polypeptides, regions which have been shown by random mutagenesis to be especially critical to filament elongation [7]. Patients with milder blistering often have mutations residing outside these end domains, and their mutations seem to affect lateral associations more than end-to-end associations (for review, see [13]).

Two additional diseases, epidermolytic palmpoplantar keratoderma and epidermal nevi of the EH type, have been added recently to the growing list of keratin disorders (Table 1). Palmoplantar epidermis from epidermolytic palmpoplantar keratoderma patients shows suprabasal cytolysis, and K9 point mutations have been identified in these patients [14*,15*]. Patients with epidermal nevi often have patches of affected skin with features of EH. Only these regions carry K10 or K1 mutations; areas that appear normal have wild-type keratin alleles.
Thus, epidermal nevi of the EH type is a genetically mosaic disorder of keratin.

The association between keratin mutations and cell fragility suggests that keratin filaments are important to the physical resilience of cells. The engineering of epidermal cells without keratins will be necessary to establish unequivocally whether keratin filaments impart mechanical integrity to cells, without which the cells become fragile and rupture upon physical trauma. This said, 60 different intermediate filament genes are differentially expressed in virtually all cells of the body, and it now seems likely that mutations in some of these genes may lead to cell degenerative disorders in other tissues. Recent transgenic mouse studies on a mutant neurofilament gene reveal striking parallels to the motor neuron disease amyotrophic lateral sclerosis (DW Cleveland, personal communication), and humans with this disease have been found to carry mutations in their neurofilament genes [17**].

Epidermal cells in association with their environment: making the connections

Basal cells adhere to the underlying basement membrane and dermis via specialized adherens junctions called hemidesmosomes (Fig. 1; for review, see [18]). Hemidesmosomes contain unique anchoring proteins, including the α6β4 integrin heterodimer and several proteins, such as BP230 and BP180, identified by autoimmune antibodies from sera of patients with bullous pemphigoid (Table 1). Inside the cell, hemidesmosomes connect to the keratin network, and it has been suggested that they do so via the unusually large cytoplasmic tail of the β4 integrin [19]. Outside the cell, hemidesmosomes connect to the basement membrane through anchoring filaments, which are composed of special epidermal laminins. Recently, mutations in one of the three epidermal laminin chains have been identified in patients with junctional epidermolysis bullosa, a recessive blistering disease caused by a loss of anchoring
filaments [20**,21**] (Table 1). The basement membrane attaches to the underlying dermis through larger cables, or anchoring fibrils, composed of collagen VII (Table 1; Fig. 1). Mutations in this gene have been detected in the DNA of patients with recessive and dominant forms of the blistering disorder dystrophic epidermolysis bullosa, and these may account for the loss of anchoring fibrils in these patients ([22**] and references therein).

Epidermal cells associate with their neighbors through calcium-activated membranous plaques called desmosomes (see Fig. 1). Despite their ultrastructural similarities, hemidesmosomes and desmosomes are composed of distinct proteins. Desmosomes contain desmogleins (Dsgs) and desmocollins (Dscs), which are members of the cadherin superfamily. Dsg2 and Dsc2 are expressed in basal cells, Dsg3 and Dsc3 are found predominantly in the lower spinous layers, and Dsg1 and Dsc1 appear to be most abundant in the upper spinous layers ([23]; for nomenclature, see [24]).

Whereas most integrin- and cadherin-mediated adherens junctions attach and transmit their signals through the actin cytoskeletal network, epidermal hemidesmosomes and desmosomes connect to keratin filaments. For desmosomes, the connection involves the carboxy-terminal ‘tail’ domain of desmoplakin, a large cytoplasmic protein sharing sequence homology to BP230 ([25**] and references therein). The amino-terminal ‘head’ of desmoplakin has been implicated in association with the plaque [25**]. How desmogleins and desmocollins form the membrane plaque is not yet clear; however, a chimeric protein composed of the transmembrane domain of connexin, a gap junction molecule, and the cytoplasmic tail of Dsg1 results in a dramatic loss of desmosomes in transfected epithelial cells [26**].

*K9 is exclusive to palmar plantar epidermis. The epidermal layers are illustrated in Fig. 1.

Table 1. Epidermal differentiation and disease.

<table>
<thead>
<tr>
<th>Epidermal compartment or component</th>
<th>Characteristic</th>
<th>Associated diseases</th>
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<tbody>
<tr>
<td>Stratum corneum.</td>
<td>Filagrin.</td>
<td>Epidermolysis hyperkeratosis (autosomal dominant mutations in K1 and K10), epidermolytic palmar plantar keratoderm (autosomal dominant mutations in K9*), and pemphigus foliaceus (auto-antibodies against Dsg1).</td>
</tr>
<tr>
<td>Granular layer.</td>
<td>Profilagrin and loricrin.</td>
<td></td>
</tr>
<tr>
<td>Upper spinous layer.</td>
<td>K1, K10, K2e, K9*, Dsg1, Dsc1, and involucrin.</td>
<td>Epidermolysis hyperkeratosis (autosomal dominant mutations in K1 and K10), epidermolytic palmar plantar keratoderm (autosomal dominant mutations in K9*), and pemphigus vulgaris (auto-antibodies against Dsg3).</td>
</tr>
<tr>
<td>Lower spinous layer.</td>
<td>K1, K10, K9*, Dsg3, Dsc3, and involucrin.</td>
<td>Epidermolysis hyperkeratosis (autosomal dominant mutations in K1 and K10), epidermolytic palmar plantar keratoderm (autosomal dominant mutations in K9*), and pemphigus vulgaris (auto-antibodies against Dsg3).</td>
</tr>
<tr>
<td>Lamina densa.</td>
<td>Type IV collagen, and fibronectin.</td>
<td></td>
</tr>
<tr>
<td>Anchoring fibrils.</td>
<td>Collagen VII.</td>
<td>Dystrophic epidermolysis bullosa (recessive collagen type VII mutation).</td>
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</tbody>
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contrast, a similar connexin chimera containing the cytoplasmic tail of Dsc1A redirects the keratin network to membrane sites containing this chimeric protein. Also localized at these new junctions is plakoglobin, a relative of β-catenin which is involved in mediating the link between cadherins and actin networks. Thus, functionally, the desmogleins and the desmcollins appear to be distinct.

Several skin diseases involve abnormalities in desmosome-mediated cell–cell adhesion. Patients with pemphigus vulgaris develop autoimmune antibodies against Dsg3 [27], and those with pemphigus foliaceus have antibodies against Dsg1 (28) and references therein). The antibodies block cell adhesion and cause severe blistering in these patients. Finally, Hailey–Hailey and Darier’s diseases both display a loss of desmosomes, leading to painful cracks in the skin. Although the genetic bases of these diseases are not known, the defect for Darier’s disease has been mapped recently to the distal end of the long arm of chromosome 12, a site that is not known to contain any of the keratin genes or the genes for desmogleins, desmocollins, desmplakin, or plakoglobin ([29] and references therein).

In addition to desmosomes and hemidesmosomes, there are also classical integrin- and cadherin-mediated adhersions junctions that attach to the actin cytoskeleton in epidermal cells. Little is known about the relationship between these two cytoskeletal-based types of junctions. It is also a mystery as to why they might both be necessary for epidermal architecture and/or for the ability of epidermal cells to sense and react to their environment. This said, most integrins are down-regulated as epidermal cells commit to terminally differentiate, and this process may be regulated at least in part by cadherins, which continue to be expressed in elaborate patterns during differentiation [30]. Thus, it seems likely that adhesions junctions play a major role in controlling differentiation and are dynamic structures, able to disassemble and in some cases reform, in response to wound healing and/or differentiation.

Final steps in terminal differentiation

In the granular layer, cells undergo a final tailoring in transcription, producing profilaggrin, a protein with potential calcium-binding sites (Table 1; Fig. 1; [31,32] and references therein). As cells become permeable to calcium, profilaggrin is processed to produce filaggrin, a protein which may be involved in bundling keratin tonofilbrils into even larger cables, called macrofilbrils. Macrofilbrils may protect keratins against the destructive phase which soon ensues. Several other changes occur in the later stages of differentiation. Membrane-coating granules, made earlier, fuse with the plasma membrane and release lipids to seal the spaces between granular and stratum corneum cells. In addition, glutamine- and lysine-rich proteins are deposited on the inner surface of the plasma membrane. Some of these proteins, such as involucrin, are made early during differentiation, others, such as loricrin, are synthesized later (Table 1; [33] and references therein). During the destructive phase, the calcium influx activates epidermal transglutaminase, which then catalyzes formation of ε-(γ-glutamyl)lysine isopeptide bonds [34]. The envelope proteins are thereby cross-linked into a cage to contain the keratin macrofilbrils. As lytic enzymes are released and remaining vestiges of metabolic activity conclude, flattened squames—mere cellular skeletons packed with keratin macrofilbrils—are released from the skin surface.

Maintaining proliferative capacity in the skin: where are the stem cells?

Through life, the epidermis must maintain a balance of dividing and differentiating cells; it must also be able to adjust this balance upon injury. Consequently, a population of epidermal stem cells must reside in skin. The search for these cells has been aided by analysis of newborn human foreskin keratinocyte cultures, which contain three clonal subtypes, holoclones, meroclones, and paraclones, possessing respectively decreasing proliferative potentials (reviewed in [1]). Holoclones contain the highest levels of β1 integrin and adhere most strongly to type IV collagen and fibronectin, a feature of basal epidermal cells [35**]. Although the relationship of these cells to stem cells in vivo remains to be established, their extraordinary proliferative potential (>100 doublings) imparts to holoclones the capacity to generate enough cells from a single clone to completely cover an adult human ([36] and references therein). This incredible capacity for self-renewal is likely to be a reflection of the need for the epidermis to undergo continual rejuvenation throughout life. Such qualities render the epidermal keratinocyte an attractive agent for gene therapy.

Where do the stem cells reside in vivo? In palmar and plantar skin, a thick stratum corneum protects the underlying basal layer, and populations of immature basal cells with slow cycling times have been detected in the deep rete ridges of this tissue [37]. These cells are still viewed as good candidates for epidermal stem cells. It is unclear whether epidermal stem cells from other body regions occupy specific positions within the basal layer. The issue is complicated by the fact that in hairless skin, follicles are able to regenerate an epidermis when it is lost from burn injury. Thus, pluripotent stem cells clearly reside in hair follicles, raising the possibility that this is a major location of epidermal stem cells in haired skin.

Similar to follicle stem cells, embryonic ectoderm is pluripotent, able to choose between hair and epidermal pathways (Fig. 3). The ability of mesenchymal condensates (dermal papilla anlage) to spatially induce follicle formation is acquired just prior to the visible wave of follicle morphogenesis, and the position, number, and
morbidity of follicles is dependent upon the rostro-caudal origin of the mesoderm (for review, see [38]). In the adult follicle, matrix cells maintain contact with the dermal papilla and appear to be a transiently amplifying population [37]. These cells can commit to at least six different modes of upward differentiation, producing concentric rings of morphologically distinct cells that include the central hair shaft, surrounded by a cuticle and an inner root sheath (Fig. 3) [38]. The outer root sheath encases the inner root sheath. The outer root sheath cells resemble basal epidermal keratinocytes and merge with them at the skin surface.

Several recent studies have begun to shed light on the elusive location(s) of hair follicle stem cells. For rodents, slow cycling and relatively undifferentiated cells reside in the bulge, a region at the midpoint of the follicle, at which the arrector pili muscle attaches (Fig. 3) [37]. This region also contains 95% of the keratinocyte colony-forming cells isolated from rat vibrissae, further

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**Fig. 3.** Structure of a hair follicle. In the growth phase of the hair cycle, cells from dermal papilla interact with proliferating matrix cells. Under an as yet unidentified trigger, matrix cells cease to divide, begin to migrate upward, and commit to six concentric rings of differentiated states; the inner root sheath, consisting of Henle’s layer, Huxley’s layer, and outer cuticle, guides the hair shaft as it emerges from the immature cortex cells, the shaft consisting of the inner cuticle and medulla. The outer root sheath is contiguous with the basal epidermal layer and has a proliferating compartment distinct from matrix cells; these cells move upward and inward as they grow and differentiate. The bulge (see text) is a possible silo for stem cells. The hair follicle shown on the right is from skin of a transgenic mouse carrying a fusion of the K5 promoter with the β-galactosidase gene. Note β-galactosidase activity in the basal cells of the epidermis, the outer root sheath, the sebaceous gland, and the bulge.
strengthening the notion that these are bona fide stem cells [36*]. The location is attractive, because the bulge effectively resides outside the hair follicle, a structure which periodically degenerates. Cyclic stimulation of new stem cells in the bulge could then take place as the hair regresses upward, perhaps bringing dermal papilla cells with it [37].

Recent studies by Rochat et al. [36*] suggest that for human scalp hairs, the bulk of the colony-forming cells reside somewhat below the arrector pili muscle attachment site, perhaps in the outer root sheath. Given that the bulge is not visualized readily in adult hair follicles, it is difficult to assess whether this site for putative stem cells is truly distinct from the bulge, or simply somewhat lower in residence than in rat vibrissae. Wherever the location, it would seem that follicle stem cells need a place to be safely tucked away during the degenerative phase of the hair cycle.

A number of questions remain. Does the same population of stem cells give rise to both matrix cells and outer root sheath cells? Does the bulge, or its equivalent, also serve as a natural reservoir for epidermal stem cells, at least in hairless skin? Unequivocal answers to these questions are likely to depend upon the ability to recapitulate hair follicle differentiation in a tissue culture system, something that despite considerable interest has eluded scientists for many years.

Molecular controls of gene expression in the skin

Regulation of epidermal gene expression

A knowledge of the major transcription factors controlling epidermal gene expression is of central importance in the effort to elucidate the molecular mechanisms underlying keratinocyte specificity and epidermal differentiation. Among the best candidates implicated in orchestrating epidermal gene expression is the sequence 5′-GCTGCGAGG-3′, first identified 5′ from the TATA box of vertebrate K14 genes [39,40]. Functionally, the sequence acts in conjunction with a distal element to control K14 transcription in cultured keratinocytes [39]. Epidermal nuclear extracts contain transcription factor(s) that bind to this sequence, which turns out to be a binding target for AP-2 [39,41]. AP-2 binding sites have now been found in the promoters of most epidermal genes and, where tested, have been shown to be functionally important for gene expression ([39–42] and references therein).

Multiple forms of AP-2 mRNA appear during skin embryogenesis ([41] and references therein; [43*]), and an AP-2 cRNA recognizing these forms hybridizes to tissues of ectodermal and neural crest lineages [44]. Using in situ hybridization to whole mouse embryos, it has been discovered recently that AP-2 mRNA patterns mimic closely, and precede slightly, those of K5 and K14 mRNAs (Fig. 2) [3**]. In contrast, a specific probe for AP-2B, a dominant-negative inhibitor of AP-2 [43*], localizes primarily to underlying mesenchyme [3**]. These studies indicate that AP-2-like mRNAs are positioned temporally and spatially to play a significant role in controlling basal epidermal gene expression during development and differentiation.

The extent to which AP-2 might be central in controlling epidermis-specific gene expression is not yet known; however, transient transfection assays have shown that recombine AP-2 can impart to hepatocytes the ability to express K5 and K14 promoter-driven transgenes in culture [3*]. AP-2B counteracts these effects. These studies do not imply an obligatory correlation between AP-2 and keratin gene expression, and it has been demonstrated that the mere presence of AP-2 does not mandate K5 and K14 expression [39]. This said, these studies do suggest that the AP-2 sequences that hybridize to embryonic ectoderm are probably reflective of an overall balance that is positive rather than negative for AP-2 activity, and that AP-2 is likely to play a significant role in K5 and K14 regulation in vivo, both with regards to its presence in basal-like epithelial cells and with regards to its absence in some other cell types.

Also enriched in epidermis are members of the AP-1 family of transcription factors, including JunB [45] and c-Fos ([46] and references therein) in postnatal skin and FosB in embryonic epidermis [47]. Many promoters that are active in terminally differentiating keratinocytes contain functionally important AP-1 sites ([48*] and references therein) and recently an AP-1 site 3′ to the K1 gene has been implicated in mediating the calcium-inducible differentiation-specific expression of this gene [48*]. This result is intriguing in view of the localization of JunB and c-Fos to the differentiating layers of epidermis, and it suggests that members of the AP-1 family may be involved in differentiation-specific gene regulation in skin.

In the past few years, researchers have begun to identify transcription factors on the basis of their abundance in keratinocytes relative to other cell types. Using this approach, a basal-specific zinc finger protein, basonucin, has been cloned [49]. Although the role of basonucin in keratinocyte gene regulation remains to be determined, its expression appears to be restricted to basal cells. Similarly, several POU-specific epidermal-enriched proteins have been identified. Anderson et al. [50] have discovered a new class II POU sequence called Skn1a (related or identical to Oct-11) which is abundant in epidermis and hair follicles. Skn1a can specifically up-regulate expression of a human K10–luciferase reporter gene, implicating Skn1a as a possible regulator of terminal differentiation in keratinocytes [50].

XLPOU1, a Xenopus class III POU protein, is also expressed in adult skin [51]. XLPOU1 shares 92% sequence homology with mouse Oct-6, which is intriguing given that human Oct-6 was isolated recently using degenerate oligonucleotides to amplify POU domain sequences that
are preferentially expressed in epidermal keratinocytes [52]. Oct-6 is one of the few POU proteins known to act in either positive or negative fashions, depending on the target gene and the tissue. In keratinocytes, Oct-6 appears to have a negative role on expression of the basal epidermal keratin genes, and it is possible that Oct-6 may play a role in down-regulating these genes as cells commit to terminally differentiate [52]. Another feature of Oct-6 expression is its sensitivity to various factors, including retinoic acid (see [52] for references). In epidermis, in which expression of both POU proteins and retinoid receptors [retinoic acid receptors (RARs) and retinoid X receptors (RXRs)] vary in a cell-type and differentiation-stage manner, such a putative interplay might elicit complex modes of action.

What governs transcriptional regulation at early times in development? Taking cues from other systems, researchers have begun to investigate expression of the Hox class of transcription factors, known to specify positional information in segmentally derived structures, in skin as well as in appendages such as limbs. Although epidermis is not a segmental structure, it overlies and is influenced by a dermis which is, in part, segmentally derived. During embryogenesis, patterning and regionalization of the epidermis is likely to be under the influence of both dermal and epidermal Hox genes. An example of the former may be Four-jointed (FJ), which shows segmental expression in E8.5 dermyotomes in a pattern which parallels E9.5 expression of basal epidermal keratin genes in the overlying ectoderm ([3*+] and references therein). At later stages, mesodermal gradients of Hox proteins are formed in feather rudiments (reviewed in [53]), suggesting that positional information directing skin appendage patterning is determined by dermal Hox genes, perhaps in a fashion analogous to their mode of action in body appendages.

Hox genes are also expressed temporally in the ectodermal component of skin. An example is the neural crest induced expression of Hox-B gene family members in the branchial arches of the head [54], preceding induction of basal epidermal keratin genes. Interestingly, transient epidermal expression of additional members of the Hox-B gene family occurs later in embryogenesis and in newborn mice, suggesting a role for homeoproteins in specifying the differentiation status of epidermis [55]. This notion is strengthened by the recent discovery of an additional differentiation-activated homeoprotein, Xdh3/Dlx3 [56].

In summary, considerable headway has been made in the quest to uncover the factors and sequences that orchestrate tissue-specific gene expression in the epidermis and other stratified squamous epithelia. In the coming years, a major focus of research will be to develop a more detailed understanding of how these many different transcription factors are integrated in a program of growth, differentiation, and development. Balancing growth and differentiation: extracellular mediators of gene expression

Enough basal epidermal cells must be present to cover the skin surface and allow periodic commitment of basal cells to differentiate terminally. The balance between proliferation and differentiation must also be sensitive to the environment and must be responsive to localized injuries. We know relatively little about the precise mechanisms that fine-tune this balance; however, a number of the players in the process have been identified, and these include growth factors, retinoids, and calcium.

Epidermal growth factor (EGF) and transforming growth factor (TGF)-α are ligands for the tyrosine kinase activatable EGF receptors, located primarily on the surface of basal cells. In addition to acting as simple mitogens, these factors (as well as retinoids) influence cell migration, a feature which may at least in part account for their growth stimulatory effects. Epidermal cells autoregulate basal growth via TGF-α production, and an increase in EGF receptors and/or TGF-α levels has been implicated in tumorigenesis and psoriasis. Newborn transgenic mice that overexpress TGF-α in their basal epidermal cells exhibit some of the features typical of psoriatic skin, including scaliness and epidermal hyperproliferation [57]. Although these mice do not develop squamous cell carcinomas, they do produce benign skin papillomas upon wound healing and/or 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment [57,58]. In contrast, ablation of either the TGF-α or EGF receptor genes in mice results in no obvious change in epidermal growth or differentiation, although abnormalities in the outer root sheath and hair follicle architecture are observed [59,*60,*]. These data suggest some redundancy in the positive regulation of epidermal growth.

TGF-βs and bone morphogenetic proteins (BMPs) have inhibitory effects on epidermal cell growth. RNAs encoding TGF-β1, TGF-β2, and BMP6 are expressed in the differentiating layers of epidermis ([61] and references therein). TGF-βs have been purported to play a role in withdrawal from the cell cycle, a prerequisite for the irreversible commitment of a keratinocyte to terminally differentiate. This is supported by the effects of overexpressing active TGF-β1 in transgenic mice [62]. Intriguingly, ablation of the TGF-β1 gene in mice leads to hyperproliferation in the basal layer, with no apparent affect on suprabasal cells [63*]. Moreover, loss of TGF-βs is associated with early high-risk papillomas in mice, suggesting that TGF-βs may act as anti-tumor promoters in skin [63*].

Although a balance between DNA synthesis and withdrawal from the cell cycle is clearly inherent in the epidermis, other factors do influence the biochemical program of terminal differentiation, and of these, retinoic acid and calcium are the two most extensively studied. Retinoic acid at high concentration (10^-6 M) has long been known to have inhibitory effects on epidermal differentiation, many of which are transcrip-
tionally regulated [4,64]. Determining the mechanism of action of retinoids on epidermal differentiation has been hampered by the number of intracellular regulators of retinoids. In addition to cellular retinol binding protein (CRBP) and cellular retinoic acid binding protein (CRABP), thought to play a role in the storage or transport of retinoids, keratinocytes express a number of the retinoid receptors that have sequence homologies to steroid receptors. These include RARα, RARγ, and RXRα ([65] and references therein). RARγ and RXRα are expressed in neonatal skin and in only a few other organs, whereas RARα is more broadly expressed. Developmentally, RARγ mRNAs appear in epidermis prior to RXRα mRNAs, and in neonatal skin, RARγ mRNAs are most abundant in the keratinizing layers of epidermis.

The control of gene transcription by RARs and RXRs is complex, involving a multitude of both indirect and direct mechanisms (for review, see [66]). RARs can heterodimerize with thyroid hormone receptors and with RXRs, and at least some of these interactions change the DNA affinity and activity of RARs. RARs can bind to thyroid-response elements, retinoic acid response elements, and retinoid X response elements, and the repertoire of complex interactions is further expanded by the capacity of RARs and RXRs to interact with AP-1 proteins. The direct binding of RARs to epidermal genes has not yet been demonstrated, although retinoic acid mediated biochemical changes in differentiation do appear to involve DNA sequences in epidermal genes that are responsive to retinoids [64].

Another regulator of epidermal differentiation is calcium. When cultured in medium containing low (<0.1 mM) calcium concentration, murine keratinocytes grow as a monolayer because desmosome assembly is inhibited (reviewed in [18]). Upon raising the calcium concentration, desmosomes form and cells stratify, TGF-β2 mRNAs are up-regulated, and a sequential induction of markers for terminal differentiation is seen ([67** and references therein). In vivo, a gradient of increasing calcium concentration is found from the spinous layer to the cornified layer, and when coupled with the in vitro data, this suggests that terminal differentiation is linked to calcium levels. Recently, it was discovered that these effects of calcium are mediated by the protein kinase C signal transduction pathway which effects gene expression of differentiation markers [67**].

In many cases, calcium seems to have a more pronounced effect on the regulation of differentiation-specific changes in cellular architecture than it does on transcription. Thus, for example, when human cells are cultured in low calcium medium, withdrawal from the cell cycle and involucrin synthesis still occur. Similarly, although desmosomes cannot assemble in low calcium, desmosomal proteins are nevertheless synthesized in both low and high calcium media [1*,18]. Finally, although changes in calcium concentrations do not seem to have a major effect on filaggrin expression in human keratinocytes, the leader peptide of profilaggrin has a calcium-binding domain, suggesting that calcium plays an important role in the formation of keratohyalin and/or the subsequent processing of profilaggrin to filaggrin [31,32]. Hence, as for other known regulators of epidermal differentiation, calcium seems to have pleiotropic effects.

Conclusions

Since the development of epidermal cell culture systems nearly 20 years ago, researchers have characterized many of the structural proteins that are intricately involved in creating the epidermis, a tissue with a capacity to withstand the physical and chemical traumas of the environment. A detailed map of when and where these epidermal proteins are expressed in development and differentiation, coupled with an understanding of the biology and biochemistry of these molecules, has shaped the foundation that led to recent discoveries of the genetic bases of many epidermal diseases. With further molecular dissection of the epidermal keratinocyte, additional discoveries in the human genetics of skin disease are undoubtedly forthcoming.

The epidermis and its associated appendages, such as hair follicles, can be seen as a series of communicating compartments with differing proliferative capacities and progressive differentiation states. Each compartment produces characteristic structural proteins and adhesion/communication complexes compatible with the location of the compartment and its role in maintaining a continually renewing structure which must provide a protective barrier function. Given the well established pluripotency of the ectoderm during early development and the capacity of hair follicles to regenerate epidermis in wound healing, the search for epithelial stem cells in the skin has been a major challenge, and one which has still not been fully resolved.

We are left with a picture in which an epidermal/hair follicle stem cell differentiates and establishes a balance of growth and terminal differentiation as a consequence of a series of environmental signals and biochemical checkpoints. These influences come from many different places and include mesenchymal signals, nutrients, and environmental messengers. Some of these pathways have been well characterized, but others remain almost completely unexplored. As future studies are conducted, the complex networks of regulatory cues that govern growth and differentiation in the skin will continue to be traced to their embryonic origins.

Note added in proof

Three papers appeared recently in the Journal of Investigative Dermatology reporting linkage of the keratin type II
gene cluster with mild cases of epidermolysis hyperkeratosis and the identification of keratin 2e mutations in patients with this disease [68–70].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- **of outstanding interest


A recent review by the major contributor in the field of epithelial integrin biology.


Reviews recent genetic, physical, and structural studies on the family of intermediate filaments.


In this paper, the induction of epidermal genes during embryonic development is examined using whole-mount in situ hybridization to uncover novel patterns of gene expression over the embryonic surface. In addition, it is shown that the transcription factor AP-2 is found at the right place and time to play a major role in the expression of basal epithelial genes.


Sequence analyses identifying mutations in the K9 gene in epidermolysis palmpantar keratoderma patients.


Sequence analyses identifying mutations in the K9 gene in epidermolysis palmpantar keratoderma patients.


Sequence analyses showing that genetic mosaicism in the K10 gene is the underlying cause of the clinical mosaicism in three different patients with epidermal nevi of the epidermolysis hyperkeratosis type.


Sequence analyses showing that mutations in one of the intermediate filament genes of neurons occur in patients with amyotrophic lateral sclerosis, a degenerative disorder of motor neurons that bears resemblance to epidermal bullous simplex.


Molecular mutagenesis studies that reveal a potential interaction between the long cytoplasmic tail of beta 4 integrin and the keratin cytoskeleton.


Genetic mapping and sequence analysis linking the blistering skin disease junctional epidermolysis bullosa to mutations in the laminin gene.


Sequence analyses identifying mutations in the laminin gene in patients with the blistering skin disease junctional epidermolysis bullosa.


Sequence analyses identifying mutations in the type VII collagen gene in patients with the blistering skin disease dystrophic epidermolysis bullosa.


24. Buxton RS, Cowin P, Franke WW, Garrod DR, Green KJ, King IA, Koch PJ, Magee AI, Rees DA, Stanley JF, Steinberg MS:


In this, and a previous paper, the authors provide evidence suggesting that an association between keratin filaments and desmosomes is mediated by desmplakin. Specifically, the head domain of desmplakin associates with the plaque, whereas its tail domain associates with the keratin network.


The authors employ a novel experimental approach utilizing chimeric targeting protein probes to examine the function of protein domains in specific subcellular locations. The technique is illustrated dramatically when a subdomain of a desmosomal cadherin is targeted to the plasma membrane, apart from endogenous desmosomal plaques. Here, the chimeric protein recruits other desmosomal components and the keratin network to this new site.


Genetic mapping data in this and related papers shows that the gene for Darier's disease maps to a region of chromosome 12 apart from the loci for known keratin and desmosomal genes.


The authors extend their many previous studies on integrins, this time showing that cadherins influence the down-regulation of integrin expression during epidermal differentiation.


A positive correlation is detected between adhesiveness, expression of integrins, and proliferative potential of epidermal keratinocytes. This represents the most successful attempt to date to define a biochemical marker for epidermal stem cells.


The authors apply a previously defined cell culture protocol, which classifies cells on the basis of their proliferative ability, to segments of human hair follicles. They identify a reservoir of cells below the bulge that have high proliferative potential and that have characteristics of stem cells.


41. Snape AM, Winning RS, Sargent TD: Transcription Factor AP-2 is Tissue-Specific in Xenopus and is Closely Related or Identical to Keratin Transcription Factor 1 (KTF-1). Development 1991, 113:283-293.


This paper presents evidence that the transcription factor AP-2 is actually a family of proteins. The authors identify an alternative splice product, AP-2b, which acts as an inhibitor of AP-2 transactivator function.


The first conclusive evidence that calcium-induced differentiation-specific gene expression in epidermis is mediated through an AP-1 element.


54. Hunt P, Wilkinson D, Krumlauf R: Patterning the Vertebrate Head: Mxgene Hox 2 Genes Mark Distinct Subpopulations of
Differentiation and gene regulation


See [60].


This paper and [59] describe the generation of null TGF-α mutant mice and report massive derangement of hair follicle organization but, surprisingly, little impairment of interfollicular epidermis or the wound-healing process. In view of the known involvement of TGF-α in epidermal proliferation and cell migration, the authors invoke a functional redundancy with unknown factors to explain the lack of epidermal disruption.


Ablation of the TGF-β1 gene in mice leads to increased proliferation in basal epidermal cells. The correlation between reduced TGF-β1 levels and increased proliferation is also seen in certain mouse skin papillomas.


First demonstration that activation of protein kinase C counteracts calcium-mediated induction of differentiation-specific gene expression, implicating the protein kinase C phospholipase C signal transduction pathway in mediating late-stage terminal differentiation specific gene expression in skin.


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